The Immunology of Pregnancy with Reference to Clinical Obstetrics

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THE IMMUNOLOGY OF PREGNANCY

WITH REFERENCE TO CLINICAL OBSTETRICS

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The curious success of the fetus as an allograft remains a mystery despite years of research. The mechanisms by which it avoids the inevitable immunologic attack and rejection expected for any genetically non-identical, histo-incompatible, or "non-self" tissue in close proximity with the host are as yet unclear. The sophistication of a system which can permit the gestation of even inter-species pregnancies (mules, ligers) yet leave the mother immunocompetent towards the challege of infection or attempts at other allografts is truly remarkable.

A large literature dealing with this subject has been developed over the years, partly, it seems, from a fascination over this clear exception to a rule of nature, and partly because of the widespread applications to clinical medicine which might be realized by an understanding of this phenomenon. Could the mechanisms be harnessed, the applications in organ transplant without generalized immunosuppression are obvious. Could the mechanisms be understood, they might well provide some insight into the success of cancerous cells, which invade normal tissues with immunologic impunity despite metamorphosed, and hence foreign surface antigens.

There is also some evidence that defects in, or side effects of the immunologic protection of the fetus may play an active role in clinical obstetrics. Indeed, the empiric or symptomatic treatments standard for such common obstetric problems as certain types of infertility, toxemia, and certain post partum complications may in fact be ineffectual or in some cases counterproductive when considered in light of current immunologic theory.

Unfortunately, human pregnancy does not lend itself well to laboratory investigation and clinical intervention for obvious moral and medico-legal reasons. There is a paucity of appropriate animal models of human pregnancy for immunologic study as few species produce a hemochorial placenta (maternal blood in direct contact with placental chorion). Non-mammals do not face the same immunologic dilemmas. Extensive knowledge of identifiable surface antigens is vital to the evaluation of inter-strain pregnancies. For the above reasons, almost all work in the field has been done with mice, which unfortunately do not suffer from certain complications of pregnancy thought to have immunologic bases.

In the sphere of human investigation, certain patients on pharmacologic immunosuppression because of other disease processes provide a model for study complicated, however, by the very disease processes for which treatment was instituted. Experiments of nature in patients with congenital

immune deficiency states are sparse as these patients, because of chronic illness and early death, rarely if ever become pregnant.

This paper will attempt to review the literature regarding altered immunologic function during pregnancy and theories of the physical, chemical and immunologic factors responsible, both those of historical interest and those of current validity. Finally, a consideration of several problems in clinical obstetrics for which there is evidence of immunologic participation will be presented, and possible modifications in therapy based on these suppositions will be suggested.

A familiarity with the basic processes of gestation will be assumed.

To protect itself from immunologic attack by the host, the feto-placental unit may utilize either, or both, of two strategies. The first is to prevent the sensitization of host's cellular and humoral immune systems by preventing contact between its tissue antigens and the host's blood-stream and lymphatic components. Failing that, the feto-placental unit must must defend itself against, or defuse any attacks launched. The methods used by the conceptus for the achievement of these ends may for the purpose of discussion be loosely divided into mechanical, hormonal/chemical, and purely immunologic mechanisms. Ideally, the placenta, while maintaining adequate contact with the mother for physiologic and nutritional support, would provide barriers to the infiltration of the fetus by maternal lymphocytes, avoiding the development of humoral antibodies and colonies of sensitized T cells, barriers to any anti-fetal antibodies which might develop, and barriers to prevent the escape of fetal or placental particles into the maternal circulation.

In the crudest of experiments--that of depriving the fetus of the barrier of the placenta by the surgical establishment of direct anastomoses between maternal and

fetal circulations in inbred and outbred rabbits--there was no difference in survival between the allogenic and syngenic fetuses. Unfortunately from the point of view of the immunologist experimenters, all fetuses succumbed to a plethoric condition thought to be due to hemodynamic disturbances resulting from increased pressures in the maternal circulation being transmitted to the delicate fetal circulation. However, the experiment was of interest in that the outbred fetuses showed no evidence of acute immunologic attack in the roughly eighty hours before the hyperemic death ensued. (Scott et al, 1973)

For years, the zona pellucida was thought to represent a sturdy mechanical barrier against immunologic attack in the pre-implantation stages of development. However, current standard techniques in embryo transfer involving the enzymatic removal of the zona demonstrate no compromise in implantation success even in third party, unrelated foster mothers.

The process of implantation includes the formation of a decidual response at the site of initial blastocyst attachment. It is a mass of fibrous connective tissue in which the cells are linked by tight junctions which surrounds the site of implantation. Various hypothetical roles have been assigned it--the possible initial channel for nourishing the blastocyst, as the cells appear metabolically very active on electron microscopy (Finn, 1971), a protective response ellicited in the uterus to prevent

invasion by an agressive trophoblast, or a protective reaction induced in some fashion by the blastocyst for shielding itself from maternal lymphocytes and the development of maternal lymphatics at the site of attachment. The lymphatic theory was given some strength by the discoveries that decidual tissue gives no protection to model fetuses (intrauterine allogenic skin grafts) when the host has been pre-immunized against the antigens of the graft tissue, (Beer et al., [illegible] 1975) and lymphatic-free pedicles enhance graft survival (Wustrack, et al, 1975).

Certain new evidence has come to light involving the roles of estrogen, histamine, and a possible contribution of the maternal immune system in the establishment of a deciduoma in the rodent. Given the data that various stimuli including the presence of the blastocyst, local minor trauma, local application of estrogens, and local application of histamine (De Feo, 1962), that local trauma and estrogen application may cause histamine release (Spaziani and Szego, 1959), that decidualization may be blocked by antihistamines (Shelesnyak, 1952), that the preimplantation blastocyst has been found to contain substantial amounts of estrogenic compounds (Borland, et al, 1977), and that uteri depleted histamine releasing mast cells by freezing do not produce deciduomata and do not implant blastocysts (Ferrando and Nalbandov, 1968), it might be hypothesized that the blastocyst, either by antigenic stimulation of a localized "anaphylaxis" with resulting histamine release, or by local

release of estrogens resulting in mast cell degranulation and histamine release, might cause the induction of decidual formation. Carrying this hypothesis further, though perhaps to an innappropriate degree, it might be hypothesized that a genetically foreign blastocyst, by exciting a larger decidual response, might pave the way for the larger placenta typically seen in cross-strain matings (see below).

Another mechanical means by which the conceptus may be immunologically shielded is the glycocalyx, a layer of highly sulfated sialomucin lying between trophoblast and uterine endometrium, (Bradbury, et al, 1969). This might work by three mechanisms: as a physical barrier preventing pieces of trophoblast from washing off into the maternal circulation and activating the maternal immune system (though there is much evidence that pieces do escape, lodging in the lung and being destroyed there by mechanisms which are not entirely clear), (Atwood and Park, 1961), as a highly negatively charged electrostatic barrier to the negatively charged maternal lymphocytes patrolling the area, (Currie and Bagshawe, 1967), or by the direct masking of surface antigens, demonstrated perhaps by the fact that attack of the mucopolysaccharide coat of trophoblast cells with neuraminidase results in the expression of strong histocompatibility antigens of transplantation (Currie, et al, 1968). The antigenicity of the trophoblast will be discussed further below.

Various hormonal substances have been hypothesized to act as generalized immunosuppressants in the maternal corculation. Blood levels of adrenal cortex hormones may be minimally elevated during pregnancy, but not to a sufficient degree to explain the success of an allograft. There is no evidence that corticosteroid-like substances are produced locally by the trophoblast producing higher concentrations which might be therapeutic. The immunosuppressive role of elevated blood levels of estrogens and progesterones have also been questioned, and the delay of first and second set skin allograft rejection by high doses of estradiol has been demonstrated, though progesterone proved ineffective (Simmons, et al, 1968). Once again however, the relatively low levels in pregnancy make this doubtful in importance as an immunosuppressive factor.

Although there is some evidence of a degree of depressed maternal immunocompetence during pregnancy as evidenced by increased susceptibility to certain viral and bacterial pathogenic organisms, (St. Hill, et al, 1972), the existence of a locally immunosuppressive compound situated at the maternal-placental junction would seem more advantageous to the host. A prime candidate for this role is human chorionic gonadotriphin (HCG), a product of pregnancy produced by the syncytiotrophoblast in large quantities at the junction between the trophoblast and the maternal circulation, producing a blood level peak during

the first trimester. Its role in reproduction remains a mystery, however, HCG has been show to inhibit in vitro both PHA induced transformation of lymphocytes (Contractor&Davies, 1973) and the mixed lymphocyte reaction (Jenkins, et al, 1972) at concentrations approximating the blood levels encountered at the peak of production in the first trimester, and of course should a local mechanism be postulated, the experimental levels would be far exceeded at the trophoblast itself.

The phenomenal success of choriocarcinoma, a malignant neoplasm of placental origin producing huge quantities of HCG and evoking no "foreign body" reaction in host tissues is an excellent experiment of nature in the evaluation of this problem. It is interesting to postulate reasons for the success of methotrexate therapy in this disease, as this drug cannot be said to be curative or achieve nearly total cell kill in any other malignancy. Perhaps instead of direct cytotoxicity, it is the "side effects" of impaired RNA and protein sythesis which are responsible for its therapeutic success, in that deprived of the ability to synthesize and enrobe themselves in HCG, the tumor cells are susceptible to immunologic attack. Clearly, as allogenic cells, they would be even more susceptible to host defences than an ordinary syngenically derived malignancy. Perhaps in following HCG levels as a measure of chemotherapeutic success, we are observing a primary process, to be followed

by immunologic cell kill, rather than the decreasing products of a dwindling cell population.

Another finding which increased interest in the role of HCG as a local immunosuppressive was the discovery that the pre-implantation blastocyst itself produces substantial amounts of an HCG-like substance which coats its surface, as demonstrable by immunofluorescent staining techniques (Wiley, 1974). As the initial implantation of the blastocyst, unprotected by barrier tissues, is one of the most difficult aspects of the immunology of pregnancy to comprehend, this discovery was of particular interest. However, as HCG levels in the blood drop sharply in the last two trimesters of pregnancy, other mechanisms must doubtless be postulated.

In the search for other substances unique to pregnancy with possible immunosuppressive effects, the Pregnancy Zone protein (PZ) came to light, though it has been espoused chiefly in Sweden. In 1959, O. Smithies at the University of Toronto observed in his exhaustive experiments with starch gel electrophoresis of serum proteins, the presence of a protein found only in the serum of pregnant women (Smithies, 1959). It was later shown to be a high molecular weight (359,000) $alpha_2$ globulin, usually found in pregnancy, but also in females taking oral contraceptive agents and in men treated with estrogens for advanced prostatic carcinoma (Margolis and Kenrick, 1969; Cooper, 1963).

Unlike HCG, which tapers off in the last two trimesters of pregnancy, PZ, detectable first at approximately eight weeks, continues to rise in concentration over the course of pregnancy, occasionally peaking the day following delivery, and then falling swiftly to undetectable levels (von Schoultz, 1974).

The usefulness, if any, of the PZ protein is as yet unclear. It has been shown to be an immunosuppressive agent, inhibiting both PHA induced lymphocyte stimulation and the mixed lymphocyte reaction (von Schoultz et al, 1973; Damber et al, 1975).

In the normal pregnancy, PZP is detectable in 25% of cases tested in gestation weeks 7-8 and in 88% tested in weeks 17-18. Two thirds of all spontaneous abortions occuring after pregnancy has been detected occur between weeks 9 and 12, and studies show that a woman with undetectable levels of PZP during this period of time runs a risk of spontaneous abortion thirty times as great as those exhibiting normal levels (Beckman, Beckman, et al, 1974). Other studies show a significant correlation between birthweight and PZP levels at term, however, no correlations are demonstrable between PZP levels and gestational age, placental weight, maternal age, parity, or previous abortion (von Schoultz, et al, 1974).

The authors of the above study interpreted their findings to mean that the production of PZP was dependent

cells for approximately eight hours, effective antibody supression would not occur. This suggested a more complicated mechanism than simple B cell suppression, and B-T cell interactions, in the form of inactivation of a helper T cell population or the activation of a suppressor T cell population were proposed.

In addition to mechanical and immunosuppressive regulators of maternal immune response, there are certain more purely "immunologic" mechanisms which have been theorized to explain the apparent lack of interaction between mother and feto-placental unit. The literature is complex and contradictory; a good number of ideas previously accepted have been shown to be invalid, but will be mentioned for historical interest.

As early as 1924, Little hypothesized that the conceptus was antigenically immature and hence not recognized as foreign by the mother, a supposition based on faulty work of the day. Interestingly, he also remarked on the apparent immunologic incompetence of the fetus during early pregnancy, an idea which continues to be accepted. The concept of fetal antigenic immaturity has been disproved by thousands of experiments since 1924, perhaps most simply by Woodruff in 1958 by the elicitation of a prompt immune reaction on the injection of its own fetal tissues into the flank of a pregnant mouse.

For a time, the uterus was felt to be an immunolo-

gically "privileged site", invulnerable to attack. However, the common occurrence of ectopic pregnancies intraperitoneally in the absence of decidual formation makes this highly unlikely.

The question of the antigenicity of the trophoblast is key in assessing maternal immune response to the feto-placental unit. As discussed previously, there may be various physical barriers separating trophoblast from endometrial tissue. However, there is much evidence to suggest that, at least at times, the trophoblast is highly antigenic.

Håkansson et al, 1975, demonstrated the presence of parental H-2 antigens on the surface of pre-implantation blastocysts, and their disappearance after the onset of implantation. Anti-blastocyst and anti-placental sera have been shown to visualize separately on the pre-implantation mouse conceptus, implying the existence of various stage specific, embryo specific, and syncytiotrophoblast specific antigen expressions during development (Wiley and Calarco, 1975).

Billington et al, 1977, visualized antigen loss during early trophoblast differentiation, while the inner cell mass was perceived to remain antigenic. At a later stage of development, Faulk et al, 1977 demonstrated no HLA antigens persisting on trophoblast, but instead their concentration on the stroma of the chorionic villi. A theory whereby

grafts, applied to the mother, by IgG of placental origin. Bernard, et al, 1977, demonstrated the presence of maternal immunoglobulins on the blastocyst at the time of implantation, in the uterine glands, on the trophoblast surface in increasing amounts over the course of the pregnancy, and coating the cells of the decidua and appearing as cytoplasmic granules therein. Acid elution of placenta was productive of an IgG capable of producing a blocking effect on the Mixed Lymphocyte Reaction (Revillard et al, 1976). The invocation of a blocking antibody also seems to yield an explanation for the aforementioned enhancement of renal allografts in passively immunized hosts.

Blocking antibody may also be important in the prevention of graft-versus-host disease or runt disease. GVH is most commonly seen in the recipients of bone marrow transplants when the alien lymphocytes attack the host, which they perceive as non-self, producing a clinical picture of skin rash, GI disturbances, liver dysfunction and defects in cellular immunity with infection. However, with reference to reproduction, GVH disease is characterized by the severe runting and subsequent death of young born under certain experimental, and perhaps occasionally natural, conditions. Beer and Billingham were successful in producing GVH disease experimentally in in young produced in four mating experiments: syngenic matings in which the mother was immunosuppressed and injected with foreign

cytes which on re-exposure to the antigen (or on occasion, to other non-specific antigens) produce direct cytotoxicity or attract via release of lymphokines, other types of cells and produce an inflammatory reaction. An example of a Type IV reaction is the PPD. Non reaction in the presence of known infection can indicate anergy, a condition associated with certain infections, tumors, and on occasion, pregnancy. In the laboratory, Type IV, or "cell mediated" reactions can bbe evaluated by the Mixed Lymphocyte Reaction, in which lymphocytes from two sources react to each others presence by the production of DNA.

It is the Type IV reaction which is thought to be responsible for allograft rejection, though all types undoubtedly are active to some extent. An example related to pregnancy of the role of the T cell was the demonstration of prolonged survival of implanting rat blastocysts in athymic mice (Håkansson et al, 1977).

Thus, the concept of a "blocking antibody" is very appealing for application to the immunologic paradox of pregnancy. An antibody which could mask crucial histocompatibility antigens would have the dual advantages of preventing attack by both the humoral and cell mediated systems, and preventing the formation of colonies of sensitized lymphocytes.

An in vivo search for candidates which might be blocking antibodies was successful. In 1974, Voisin and Chouat demonstrated the enhancement of paternal origin skin allo-

nodes, which drain the uterus, in the mother prior to pregnancy. This observation, along with passive transfer studies, led to the suspicion that a humoral antibody was the principle effector of this protective maternal influence on placental growth, conceivable a "blocking antibody".

At this point, perhaps a brief review of various types of immune response applicable to allograft rejection, and a summary of certain inhibitory factors is in order. In Type I reactions, circulating antibody (IgE) binds antigenic determinants on the cell membrane as well as basophils or mast cells, causing release of vasoactive amines. In Type II reactions, circulating IgG or IgM binds to antigenic determinants on the cell membrane, leading to complement fixation and target cell destruction In cases where complement fixation does not occur, IgG may function as a so called "blocking antibody", attaching to the antigen and masking it, preventing its attack by cytotoxic antibodies or lymphocytes. In so called "antibody-dependent cell mediated cytotoxicity", cell kill depends on the presence of both antibody and non-T non-B lymphocytes or null cells. In Type III reactions, the deposition of antigen-antibody complexes in the tissues lead to cell damage at the site of deposition via complement, and the release of lysozomal enzymes, vasoactive amines, etc. In Type IV reactions, previous antigen exposure is responsible for the production of colonies of sensitized lympho-

It has long been known that outbred rodents tend to produce larger litters with heavier feto-placental units, quite opposite to what one might expect invoking the possibility of immunologic attack by the mother. The reasons for this were completely obscure until a series of experiments were performed by Beer and Billingham, leaders in the field of the immunology of pregnancy. They found that the presensitization of the rat uterus with paternal cells or washed sperm could engender a markedly increased "flare" reaction in the endometrium when it was re-exposed to paternal antigens in the form of the blastocyst. Far from decreasing fertility, more implantations were noted, each productive of a heavier conceptus. It was postulated that increased vascularity of the site of implantation led to increased penetration of the trophoblast and a larger placenta (Beer and Billingham, 1974) or that maternal antigens stimulated placental cell growth (GVH reaction).

Further experiments demonstrated that, though larger placentas were produced by allogenic fetuses than by syngenic fetuses, this difference could be enhanced by the presensitization of the mother to paternal antigens, and reduced by the induction of tolerance to paternal antigens (Beer et al, 1975).

Interestingly, it was noted that the "advantages" of outbreeding (as measured by feto-placental weight) could be much reduced by the excision of the para-aortic lymph

antigens might be polarized on the fetal side of individual cells was proposed.

Low levels of both HLA-A and HLA-B antigens (approximately 5% of levels detected on lymphocytes) were noted in mid-gestation by Goodfellow et al, 1976.

Secondary evidence of the antigenicity of the trophoblast exists in that anti-HLA antibodies are produced in response to hydatiformmole pregnancies comprised only of trophoblastic tissues (Lawler, 1974). Both normal pregnancies and ectoplacental cone implants have long been known to stimulate local lymphadenopathy in the draining nodes. Finally, the production of antibodies directed against the trophoblast has been documented. Their presence and their possible contribution to the decreased perceived antigenicity of the trophoblast will be discussed below.

Curiously, there is much evidence to suggest that fetal genetic disparity may exert a beneficial influence on the development of the conceptus. In the many experimental attempts to jeopardize the conceptus by the induction of transplantation immunity to parental antigens, there have been numerous failures (Lanman et al, 1962) and only the occasional success (Breyere and Springer, 1969), in this case demonstrating decreased litter size in mothers previously sensitized to paternally derived sarcoma cells. The phenomenon of enhanced renal allograft survival in passively immunized hosts has long been appreciated but not understood.

on a healthy conceptus. However, the data are equally consistent with PZP as being necessary for a healthy conceptus and the prevention of abortion. Unfortunately, there has been no further work in this field which sheds light of the question of the role of the PZP or the mechanism of its production.

Another possible immunosuppressive agent associated with pregnancy is alpha fetoproteir (AFP). A major constituent of amniotic fluid, it is produced by the fetal liver in large quantities during embryogenesis, levels peaking in early-mid gestation, dropping linearly with approaching birth, and falling to extremely low levels after birth which are maintained during adulthood except for certain neoplasms and diseases involving areas of regeneration in the liver. In the laboratory, AFP has been shown to inhibit both primary and secondary antibody response (Murgita and Tomasi, 1975a) as well as the mixed lymphocyte reaction and mitogen-induced lymphocyte transformation (Murgita and Tomasi, 1975b). Obviously, the timing of the appearance of AFP and its reappearance in cases of malignancy make it an appealing candidate for an immunosuppressant in both cases.

Interestingly, it was found that the timing of addition of AFP to antigen stimulated antibody producing cell cultures was vital. Were the AFP not added to the cultures early in the stimulation process and allowed to incubate with the

lymphocytes, syngenic matings in which the mother received foreign lymphocytes specifically sensitized against her strain, allogenic matings in which the mother received lymphocytes sensitized against the paternal strain, and in allogenic matings in which the mother was presensitized to paternal antigens via skin grafts. It was noted, however, that the results of this last experiment were time dependent--that if matings occured more than two weeks following graft attempts, GVH disease did not develop in the young. Similarly, had a mating occured before grafting attempts, GVH disease could not subsequently be produced in the young of any mating. This seemed to indicate the time-dependent development of a blocking antibody protective against the development of GVH disease (Beer and Billingham, 1975). In light of the fact that GVH disease is almost unknown in "unadulterated" pregnancies, although there is good evidence for the exchange between mother and fetus of leukocytes both during pregnancy and at delivery (Gill, 1977), it seems likely that similar factors are at work during normal gestation.

Various other mechanisms of "enhancement" or immune mediated suppression of graft rejection have been proposed. The importance of antigen-antibody complexes in the inhibition of in vitro cytotoxicity has been investigated, employing a number of experimental models. In a tumor model of enhancement, sera from mice carrying viral-induced sarcomas were shown to block the cytotoxic effect of lymphocytes

immune to the tumor-specific antigens of the neoplasms. By absorption onto tumor cells and subsequent elution, two fractions were recovered, neither of which alone had any blocking effects. However, a 1:1 mixture of the two fractions demonstrated blocking, as did the lower molecular weight fraction if incubated with target cells and lymphocytes, data very suggestive that an antigen-antibody complex was the active factor in the blocking activity. (Sjögren, et al, 1971). Later work by the same group revealed that the higher molecular weight portion contained IgG, while the lower did not, was presumed to be antigen, and was presumed to have its activity dependent on interaction with effector lymphocytes.

A similar study involving liver allograft rejection demonstrated prolongation of survival with the administration at equivalence of alloantigen and antibody raised against the alloantigen (Myburgh and Smit, 1975).

Another candidate for the inhibition of cell-mediated and/or humoral immune response is the "suppressor T cell". Again, in studies plucked from organ transplantation literature in the absence of experiments involving gestation, Jirsch et al, 1974 demonstrated increased cardiac allograft survival in mice treated with lymph node cells from tolerant mice, in the absence of transferred serum factors. Kilshaw et al, 1975 demonstrated a similar transfer of tolerance via spleen cells. T cells were

From the experiments presented in the first section of this paper, it may be appreciated that immunologic factors play an important and, to some extent, unappreciated role in normal and abnormal pregnancy. Certain parallels can be drawn between possible malfunctions of the proposed mechanisms and known disease states encountered in pregnancy. There is now reason to believe that in some cases, infertility, spontaneous abortion, herpes gestationis, toxemia, and pregnancy associated disseminated intravascular coagulation may have immunologic causes. These will be discussed, with reference to possible therapeutic modalities directed at the immune response.

As discussed previously, the pregnant human female is not well suited to experimental intervention and the greater part of the work in this field relies on "experiments of nature", mouse studies, retrospection, and pure theory.

Finally, a short discussion of the major histocompatibility complex with reference to ontogeny and phylogeny will be presented.

Infertility and Spontaneous Abortion

In the clinical evaluation of the patient with primary infertility, procedures are undertaken to assess functioning of the various steps necessary for the successful initiation of a pregnancy--hormonal studies to establish ovulatory function, evaluations of sperm number and motility, patency of the Fallopian tubes etc.--all points of possible breakdown, most amenable to attempts at therapeutic intervention. However, the standard infertility work-up reveals no recognizable cause in approximately 30% of patients (Dor et al, 1977). Because of the conceptus's tenuous position as allograft, it seems not unlikely that some of these reproductive failures may fall into the realm of the immunologist.

Let us propose three types of infertility based on the immunlogic concepts discussed in the first part of this paper; first, the well known "anti-sperm antibody" theory which has been espoused by certain clinicians, though it has fallen out of favor recently, second, the failure of implantation due to immunologic destruction by the very inflamatory process necessary for nidation, third, abortion soon after implantation or at any step in gestation due to the inadequate production of "blocking antibodies" and the subsequent rejection of the conceptus, or to their inability to overcome a particularly strong antigen or pre-existing antibody. Certain laboratory data support each.

presumed to be the vector as treatment with antitheta antibody and complement abolished the effect.

Thus, there is reason to believe that many factors contribute to the success of the fetus as allograft; the sine qua non of immunosuppression does, however, seem to be the blocking antibody.

The "anti-sperm" antibody theory, which caused much excitement on its introduction, states that fertility may be impaired by the development in the female of immobilizing or sperm-agglutinating antibodies in response to sensitization by sperm introduced vaginally (Franklin and Dukes, 1964). It was shown that a higher percentage of women suffering from infertility of unknown cause had circulating antibodies to sperm (72%) than did fertile women (5.7%). Certain objections to this theory have been encountered. Other studies have failed to show the same high percentages of infertile women (Glass and Vaidya, 1970). Attempts aimed at contraception via the sensitization to sperm antigens by the parenteral route have been noticeably failures (Edwards, 1960), even though this route (Tyler et al, 1968) is far more effective than vaginal absorption in stimulating antibody response. Studies ascertaining the percentage of infertile women with antibodies have used women of known fertility for controls, usually requiring at least two pregnancies. Though this seems to be done for obvious reasons, it prevents the possible association between pregnancy and decreased antibody. Were women who had avoided pregnancy by the consciencious use of, for example, birth control pills used as controls and estimated to be about 70% fertile, the possibility that "blocking antibodies" left over from a previous pregnancy prevented antigenic stimulation by deposited sperm in parous females could be

evaluated.

It has also been hypothesized that locally produced antibodies in the genital tract could prove spermicidal, and indeed, these can be shown to be produced (Lippes, et al, 1970). However, the role of such antibodies remains obscure especially in light of the previously discussed data linking local sensitization with washed sperm to increased fertility and improved fetal growth withing the sensitized uterus. It may well be that the antibodies detected by Lippes et al prove advantageous.

In general, no conclusions have been reached regarding the deleterious nature of the anti-sperm antibodies. However, it is usual practice to prescribe an empiric trial of condom therapy (preventing female contact with sperm antigens) while monitoring anti-sperm antibody levels. This method has enjoyed some degree of success (Glass and Vaidya, 1970).

Far less research has been devoted to the investigation of faulty implantation as a cause for infertility, probably because of the difficulty of these investigations in the human. It does not seem unreasonable that an initial "anaphylactic" contact with the uterine wall could prove destructive to the blastocyst. Furthermore, prior sensitization to paternal antigens, either via sensitization to blood group antigens or the aforementioned anti-sperm antibodies, could produce a secondary immune response at the site of

implantation vigorous enough to imperil the embryo. Naturally occurring antibodies such as the secretory antibodies against ABO determinants could also work at this stage. In the one existing study of implantation efficacy in the human, uterine scrapings were obtained from a typical gynecologic clinic population, some of whom complained of infertility. Careful examination of the scrapings revealed numerous persistant blastocysts which had failed to implant presumably because of the marked lymphocytic and plasma cell infiltrates in the nearby endometrium suggestive of an incompatibility response at the site of attempted nidation (Jorgensen, 1970). The precise reasons for the failure of implantation are, of course, obscure, and further study in the infertile population (rather difficult because of the possibility of inadvertent abortions) with attention to various antigenic systems is needed.

The problem of infertility due to abortion--recognized or otherwise--must be separated from infertility due to faulty implantation. While it is possible to reduce implantation via the active or passive sensitization of the mother to paternal antigens, it is exceedingly difficult, if not impossible, to cause mothers to abort alien fetuses by sensitization against paternal antigens (Beer and Billingham, 1977). While the demise of the blastocyst may be seen as due to sensitization, with the development of cyto-

toxic antibodies and antigen-specific lymphocytes, the demise of the fetus may be considered as due to the lack of sensitization, and hence the lack of production of sufficient amounts of the appropriate blocking antibodies to prevent attack by the immune system or any pre-existing antibodies. The amount of blocking antibody required to maintain a pregnancy might be assumed to be proportional to the degree of genetic disparity between mother and fetus, and in cases of extreme disparity, this mechanism might prove insufficient, resulting in abortion. The histopathology of the chromosomally normal abortus usually reveals focal areas of decidual necrosis with an intense inflammatory infiltrate suggestive of a rejection phenomenon; it is however, difficult to say whether this represents a primary phenomenon or a secondary reaction to a conceptus which has succumbed to one of the many non-immunologic, non-chromosomal causes of abortion in each individual case (Robbins, 1974).

That naturally occurring or previously acquired antibody may be harmful to the conceptus can be demonstrated by Rh and ABO incompatibility, both of which may damage the fetus. The important difference between these two systems is that ABO antibodies, which occur naturally, may damage the first pregnancy, while Rh sensitization affects further pregnancies. The deleterious effects of Rh sensitization are known to all; fewer realize the impact of the ABO system on fertility. In a study of karyotypically normal abortuses

of the first 16 weeks of pregnancy, it was shown that a significantly higher incidence of ABO incompatibility between mother and fetus was present than in karyotypically abnormal "controls" (Lauritsen et al, 1975), the maximum fraction of abortions for which ABO incompatibility might be responsible being 18%. Other studies (Tyler et al, 1967; Behrman et al, 1960) have noted the high frequency of ABO incompatibility in the population of physiologically normal infertile couples.

The role of HLA incompatibility in infertility and spontaneous abortion is as yet poorly defined. Mittal, 1975, demonstrated no decrease in pregnancies or offspring in HLA incompatible couples. In another study, twenty-nine couples with spontaneous abortions were evaluated according to the number of HLA-A and HLA-B antigens which the parents had in common and compared to couples with a successful pregnancy and no abortion history. 59% of the parents of the abortuses shared none of the possible two HLA-A antigens, while 37% of controls shared none. Although these results were not statistically significant, they are intriguing and certainly bear repetition with a larger series (Lauritsen et al, 1976). Anti-HLA blocking activity has been noted in serum. Its significance is unknown (Jeannet et al, 1977).

The role of the immune system in the 54% (Kajii et al, 1973) of abortuses which are chromosomally abnormal is unexplored. Many of these fetuses are physiologically or biochemically unfit and

succumb for reasons of their own. However, certain karyotypes which are potentially viable (eg. trisomy 21) are heavily represented in the spontaneously aborted population. Indeed, only one in five Downs' syndrome fetuses survives to birth. Although this phenomenon could certainly represent a spectrum of viability, it is perhaps worth considering whether the karyotypically abnormal fetus could be recognized as such and immunologically discarded. For this rather unlikely hypothesis to be true, antigenic incompatibility would have to be capable of causing abortion (as discussed above) and chromosomal aberrancy would have to affect surface antigens displayed by the cells. There is no evidence that the latter is the case, except in cases where a defect in the histocompatibility locus is involved. However, there is also no evidence that it is <u>not</u> the case and as chromosomal abnormalities may cause defects in protein and enzyme synthesis, the idea that surface antigens could be affected is not unthinkable. Whether they could be changed enough to over-ride the preservative influence of the blocking antibodies is another question.

Interestingly, though rather off the subject, embryos with deletions in the T locus (located on chromosome 17 next to the major histocompatibility locus, similar in makeup, and thought to code for surface antigens necessary for cell-cell identification and interaction) cease growth at specific and predictable stages in development and might

be viewed as an example whereby the nascent fetal "immune system" was responsible for its own demise (Bennett, 1978).

The effects of various immunosuppressive and immunostimulating agents on reproductive efficacy may also give some insight into the role of the immune system in the normal pregnancy. As noted previously, the mating of mice of two different strains tends to produce litters of more numerous offspring with larger feto-placental units. The reasons for this may be an increased decidual response caused by the genetically foreign material, leading to a larger placenta, and that leading to a larger fetus, or a stimulation of the placental cells by the neighboring maternal cells and a resulting increased multiplication in what might be called a mild GVH reaction, also leading to a larger placenta. Cortisone acetate has been shown to delay or inhibit ovoimplantation in the rat, although this did not appear to be due to its anti-inflammatory activity as higher equivalent doses of non-steroidal anti-inflammatory agents (indomethacin, phenylbutazone, flufenamic acid) failed to have similar effects. These drugs were, however, noted to decrease decidual response resulting from uterine trauma. A central mechanism for the action of cortisone acetate was postulated (Bennett et al, 1977).

In another study, allogenic matings were treated with one of three immunosuppressive or cytotoxic agents

(methylprednisolone, cyclophosphamide, azathioprine), one of three immunostimulants (B. pertussis, C. parvum, BCG), or saline control. Though no difference in number of implantations was noted, the immunostimulated group showed a trend toward larger feto-placental units and increased spleen and para-aortic node weight, while the immunosuppresive/cytotoxic group tended toward smaller feto-placental units and smaller spleens and para-aortic nodes. Azathiprine resulted in 100% resorption of fetuses, cyclophosphamide resulted in 50% resorption (Scott, 1977). The most interesting finding in this study was that the cyclophosphamide resorption rate could be corrected to normal (near zero) by the concommitant administration of the non-specific immunostimulant BCG, data completely consistent with the idea that immunostimulation, rather than immunosuppression is necessary for the maintenance of an allogenic pregnancy, conceivably to promote the production of blocking antibodies. The experiment would well be repeated using syngenic matings as controls.

It must be remembered that the above experiments were performed using a system which did not suffer from infertility, and therefore any benefits (permitting implantation or preventing early abortion) which might accrue in certain circumstances would not be visualized. Therapeutics in human infertility is a difficult business as the precise diagnosis--whether infertility results from failure of

fertilization, failure of implantation, or unrecognized spontaneous abortion--is always in doubt. Condom therapy has been tried, possibly with some success, in certain cases of infertility; perhaps in others the intrauterine instillation of washed sperm could raise implantation rates as suggested in the animal model. It is also not unthinkable that in certain circumstances, short courses of immunosuppressives or immunostimulants could be appropriate.

The clinical experience with the immunologic management of infertility with pharmacologic agents is almost nonexistent. Shulman, et al, 1978 have used corticosteroids in male autoimmune infertility with some success, and in female, presumably "anti-sperm antibody" infertility with a lesser degree of success. Women on chronic immunosuppressive therapy for transplantations or systemic disease have smaller feto-placental units than normals, however, the effects of their underlying disease on fetal growth is difficult to evaluate. In one case, a diseased "control" was available--a woman with end stage renal disease who received a transplant from a twin and thus required no immunosuppression. Her fetoplacental unit was of normal weight. (Scott, 1977)

Obviously, a much greater understanding of immune infertility is needed before therapeutic trials become appropriate. However, it should be considered in the differential of refractory infertility, and under certain circum-

stances, investigation of such parameters as ABO incompatibility may be in order.

Herpes Gestationis

Herpes gestationis is a rare bullous disease occuring only during pregnancy or shortly post partum, usually beginning during the second trimester. It is characterized by a pruritic erythematous vesiculobullous eruption appearing on abdomen, extremities and buttocks occasionally accompanied by systemic manifestations of malaise and fever, and striking eosinophilia.

Immunologic and histologic investigation of the disease have demonstrated the presence of the so-called "HG" factor in the serum of affected patients, thought to be an IgG. This factor deposits in the basement membrane zone between the dermis and the epidermis causing complement fixation and cell damage, resulting in the eruption. (Jordon, 1976) HGF can cross the placenta and affect the fetus which may be born with a similar eruption. There is also an increased incidence of spontaneous abortion, stillbirth and prematurity among offspring (Lawley et al, 1978).

Herpes gestationis tends to recur with subsequent pregnancies and with the use of estrogenic oral contraceptives. It generally responds to systemic high dose corticosteroids, and occasionally to local therapy. There is no correlation (contrary to what was once thought) with Rh sensitization or toxemia.

As herpes gestationis is a disease associated with an

IgG found during pregnancy, it is tempting to build a case linking it to the production of blocking antibodies. Complement fixation may occur as the result of deposition of antigen-antibody complexes or the deposition of antibody alone. As HG can recur, with estrogenic stimulation, long after birth, when presumably no placental antigens are available, it seems possible that blocking antibody, specific for the basement membrane of the placenta, could cross react with the basement membrane zone in the skin and there activate the classical pathway of complement. Although complement fixing cytotoxicity does not occur in the placenta, antibodies can fix, or not fix complement depending on circumstances, and it is possible that in the placenta the abundant carbohydrate moieties on the surface prevent exposure for the reactions necessary. This possibility is consistent with the timing of disease--either in the second two trimesters when antibody levels presumably continue to rise, or with onset shortly after birth, when the sudden removal of the target cells would result in a temporary overload of the maternal circulation and increased opportunity for deposition.

The telling experiment in this case is an easy one--the acid elution of a washed placenta from an affected mother, and the subsequent immunofluorescent staining of a skin biopsy obtained on clearing with the IgG gathered. Unfortunately, the disease is so rare a case might take years to find.

Toxemia of pregnancy and DIC

A plethora of theories on the etiology of toxemia of pregnancy have been proposed. There is increasing evidence that this disease, characterized by edema, hypertension, proteinuria, and in its worst form, seizures, may well have an immunologic basis.

Certain features of toxemia may be important in determining its etiology. It has been noted that the prevalence of toxemia is increased in the primagravida, multiple pregnancies and hydatiform molar pregnancies, in the diabetic, patients with previous hypertensive vascular disease, or previous renal disease. It progresses over the course of pregnancy and the definitive treatment is delivery of the placenta, although toxemia may first appear an hour or so after delivery. The disease is frequently associated with poor fetal outcome.

Histopathologically, toxemia is a diffuse disease effecting similar changes in many organs of the body. The vessels of brain, kidney, liver and placenta demonstrate the changes of vascular disease as well as fibrin and platelet deposition, intravascular coagulation. Perivascular hemorrhage and tissue edema are also seen; the lung may reveal pulmonary edema, intravascular coagulation and alveolar hemorrhagic diathesis similar to that encountered in endotoxic shock. The placenta shows multiple infarcts and trophoblastic degeneration.

The uteroplacental junction displays fibrinoid necrosis and narrowing of the spiral arteries in the decidua and similar changes in the uteroplacental arteries of the placental bed. The necrotic vessels harbor foamy lipophages, fibrin, macrophages, and thrombi and are surrounded by mononuclear infiltrates. (Robertson, 1975) These changes, known as atherosis, are similar to the pathology encountered in renal graft rejection.

Thus, one theory of toxemia which may be supported by much evidence is that a deficiency in blocking antibody leads to damage of the placenta causing the release of certain substances which alone, or in combination with circulating antibodies may cause damage to the vasculature and a low grade disseminated intravascular coagulation syndrome.

The evidence that toxemia may be touched off by a deficiency in blocking antibodies is strong. It is a disease almost exclusively of primagravidas, who, as mentioned in the discussion of tolerance, would have had no opportunity to be presensitized to paternal antigens except via sperm. Interestingly, numerous exposures to paternal sperm prior to conception has been shown to decrease risk of toxemia (Marti and Herrmann, 1977). Prior blood transfusion also has a protective effect (Feeney et al, 1978). Toxemia in second pregnancies is far more common when a different father is involved

(Need, 1975). A decrease in anti-HLA antibodies below usual pregnancy levels has been noted (Jenkins et al, 1977). An increased blocking load is present in multiple pregnancies and molar pregnancies which frequently are associated with toxemia.

Interestingly, except for certain anecdotal reports of toxemia in Rh incompatible pregnancies (Hirsch and Mark, 1964; Nicolay and Gainey, 1964), the disease does not seem to occur in cases of great antigenic challenge, rather, victims have increased maternal-fetal HLA compatibility over normal pregnancies and tend to have a depressed MLR between lymphocytes from maternal and cord blood (Jenkins et al, 1978). No difference is noted in the frequency of toxemia in monozygotic vs. dizygotic twins (Campbell et al, 1977) It is perhaps possible that fetuses lacking major histocompatibility complex differences fail to stimulate the production of blocking antibodies, yet are later rejected on the basis of other determinants.

As mentioned above, the placenta in toxemia shows pathology characteristic of a rejection phenomenon. Evidence that placental damage is the second step in the production of toxemia is that, much in the fashion of the narrowed utero-placental arteries causing poor perfusion and infarcts, toxemia can experimentally be reproduced by partial ligation of the uterine artery (Hodari et al, 1967). It may be argued that placental infarction is secondary to vessel

disease caused by another mechanism; it is perhaps more likely that the process is a multiplying effect whereby placental damage leads (see below) to increased vessel damage, resulting in even poorer placental perfusion.

Although the immunologic destruction of the placenta is equally consistent with a graft vs. host phenomenon, this mechanism seems unlikely in that mole pregnancies, containing no lymphoid tissue, are among the leaders in producing toxemia.

Noting the lupus-like features of toxemia, it should not be surprising that the major immunopathological lesions are the presence of circulating immune complexes and their deposition in vessel walls and the renal glomeruli (Stirrat et al, 1978; Kitzmiller et al, 1973; Morris, et al, 1964) In the vessel, complex deposition and subsequent complement fixation can lead to endothelial damage, platelet aggregation, and the activation of the coagulation system; in the kidneys, damage to vessels and glomerular basement membranes can produce a decreasing GFR, proteinuria and hypertension on a poor perfusion or volume overload basis. There is evidence that immune complex deposition occurs to some extent even in normal pregnancies (Tung, 1974), and in the presence of pre-existing renal disease, the mechanism for clearance of the Ag-Ab complexes is faulty, they linger longer and incur more destruction, possibly explaining the increased

incidence of toxemia in patients with renal lesions. Similarly, it might be proposed that in patients with pre-existing vascular disease (diabetics, hypertensives) previous vessel damage might predispose to the deposition of complexes and further destruction.

The source of the antigens in the above model is presumably the placenta, perhaps increased antigen release stimulating the production of greater amounts of antibody and increasing amounts of the complex. Alternatively, the placenta's ability to dispose of complexes, turning them into innocuous substances could be overwhelmed, as suggested by Morisada et al, 1976, leading to their dispersal into the circulation, or soluble "blocking antigens" could be released by the placenta, disarming lymphocytes and combining with antibody, a variation on a theme proposed by Halle-Pannenko et al, 1976. There are few, if any animal models of toxemia, possibly because a hemochorial placenta, rare in animals, provides freer access to the maternal circulation for placental antigens.

A subacute DIC picture plays an important part in the pathology characteristic of toxemia. Although there has been a certain amount of dissention (Condie, 1976) it seems clear that in toxemia the clotting parameters are suggestive of DIC (Bonner et al, 1971). In the infarcted placenta or the dead fetus, other factors, such as release of tissue thromboplastin undoubtedly

play a role. Indeed, a case has been reported of fetal demise being followed by severe DIC and subsequent severe toxemia (Imrie and Raper, 1977). Whether massive antigen release, tissue thromboplastin release, or both led to the toxemia is unclear.

Indeed, resembling a giant hemangioma, the placenta may be a particularly vulnerable site for the initiation of a DIC-like process. There is evidence of increased fibrinolytic activity in the normal fetal circulation, (Foley et al, 1977) which may decrease in the toxemic pregnancy fetus (Condie, 1976).

DIC or toxemia encountered post-partum may be due to the large transfer of antigenic blood components which occurs at partuition as documented by Gill, 1977. The lack of a "target organ" would assure abundant antibody supply.

A DIC picture associated with immunologic activity is reminiscent of thrombotic thrombocytopenic purpura, a disease of unclear etiology associated with lupus characterized by a microangiopathic anemia, thrombocytopenia, renal damage and neurological symptoms, in which fibrin, gamma globulin and platelets deposit in and cause microthrombosis of heart, brain, kidney, pancreas and adrenal. It is interesting to speculate on a similar but autoimmune etiology.

Previously, therapy for toxemia has been symptomatic

only, as well it might be as the etiology of this syndrome is just beginning to be elucidated. However, using the theory just presented, it is interesting to speculate on possible prophylaxis and treatment for toxemia.

In the earliest stages, it might be feasible to attempt prophylaxis or treatment with immunostimulant agents, either paternal lymphocytes as suggested by Scott and Jenkins, 1978, or non-specific immunostimulants such as BCG (which has not to date been shown to be teratogenic). Anticoagulation might be tried to prevent the low grade DIC picture, and in one case, warfarin was administered to a patient who had had two stillborn pregnancies complicated by severe toxemia. She remained normotensive until late pregnancy, when the drug was stopped, at which time her pressure rose within 24 hours. She delivered a normal child (Valentine and Baker, 1977) Warfarin was probably a poor choice because of the associated saddle-nose deformity, but heparin or an anti-platelet agent might be suitable.

The use of steroids in late toxemia has not been espoused, though presented with tissue destruction initiated by antigen-antibody complexes, complement fixation and resulting inflamation, they seem the obvious choice. In glomerulonephritis, cortisone has been shown to inhibit the transit of immune complexes across vessel walls, hence preventing glomerular injury and inflamation (Germuth et al, 1968).

A caveat in the use of steroids is that they may significantly decrease antibody production--obviously undesirable in early toxemia as a deficiency in blocking antibody may be the root of the problem. Also, because of the kinetics of antigen-antibody interaction, a decrease in antibody may actually lead to an increase in Ag-Ab complexes as the two species approach equivalence. This could conceivable lead to further destruction. (This reasoning could even be used to explain, primarily, why decreased antibody production could lead to toxemia, without invoking placental destruction from an immune reaction. However, it is more likely that steroids could be of some benefit in late stage disease when complexes threatened massive end organ damage, and could conceivably have the added benefit of minimizing any rejection reactions directed against the placenta. Interestingly, toxemia is associated with decreased serum cortisol levels compared with normal pregnancy (Galvao-Teles and Burke, 1973).

There have been few studies on the effects of corticosteroids on pregnancy, and none not complicated by underlying disease in the subject. However, in a study of 34 patients receiving corticosteroids for various reasons, including some patients with pre-existing renal disease, only one case of elevated blood pressure was noted (Warrell and Taylor, 1968). Further studies are needed to define the possible therapeutic role of steroids in toxemia. One can

well imagine the hesitation of the clinician to treat an edematous and hypertensive patient with a drug having some degree of mineralocorticoid activity.

Finally, in cases of toxemia severe enough to require delivery or interruption of pregnancy, an investigation of which techniques of delivery and abortion result in the least antigen load to the mother could be useful in the prevention of post-partum toxemia and DIC.

Teleology and the Histocompatibility Antigens

In addition to the obvious advantages of self/non-self recognition in infection and tumor invasion, the MHC may, with a certain amount of teleologic reasoning, be perceived to provide other benefits to the future of the species via the mechanisms of gestation. Genetic disparity, heterozygosity and the avoidance of the production of double recessives in consanguineous matings are evolutionarily beneficial. The previously discussed tolerance phenomenon, whereby conceptuses genetically disparate from both mother and previous conceptuses enjoy improved survival and growth, may play an important role in achieving these desirable ends. This phenomenon has been noted in sex order studies (Vernier, 1977). Primiparas tend to have enhanced survival of male conceptuses, genetically disparate with regard to H-Y antigens from the mother, while in subsequent pregnancies fetuses of the sex opposite to that of the previous pregnancy are favored.

As mentioned previously, it is possible that other mechanisms exist to weed out the genetically unfit conceptus, the immune system patrolling the uterus for the extremely deviant fetus. It is interesting to speculate if the aging immune system in older mothers (demonstrated in mice by decreased responsiveness to PHA stimulation and tumor cell challenge (Perkins and Cacheiro, 1977) might be involved in the increased numbers of genetically anomalous infants born.

Finally, self-exposure to the MHC during development of the fetal immune system may be vital in establishing the knowledge of "self" and preventing autoimmune phenomena, as may be seen in tetraparental allophenic mice in which two lymphoid cell systems live in harmony (Barnes, 1976).

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